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INCLUSION PRODUCT OF COENZYME Q_{10} IN METHYLATED-β-CYCLODEXTRIN AND PRODUCTION METHOD THEREOF

Masao Iijima et al.

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INCLUSION PRODUCT OF COENZYME Q_{10} IN METHYLATED-β-CYCLODEXTRIN AND PRODUCTION METHOD THEREOF

[Ho koso Q_{10} no mechiruka-beta-shikurodekisutorin hosetsutai oyobi sono seizoho]

Inventors: Masao Iijima et al.

Applicant: Zelia Shin Yaku Kogyo K.K.

[Amendments have been incorporated into the text of the translation.]

Claims

1. An inclusion product of coenzyme Q_{10} in heptakis(2,6-di-o-methyl)-β-cyclodextrin

2. A production method of inclusion product of coenzyme Q_{10} in heptakis(2,6-di-o-methyl)-β-cyclodextrin, characterized in that coenzyme Q_{10} is dispersed in an aqueous solution of heptakis(2,6-di-o-methyl)-β-cyclodextrin using ultrasound, followed by dissolution through agitation.

3. A hydrated inclusion product according to the description in Claim 1).
4. A freeze-dried inclusion product according to the description in Claim 1).

Detailed explanation of the invention

This invention pertains to an inclusion product of coenzyme Q_{10} (hereafter abbreviated as CoQ_{10}) in heptakis(2,6-di-o-methyl)-β-cyclodextrin (hereafter abbreviated as DM-β-CyD) and production method thereof, as well as to enhancement of the conventionally known drug efficacy-pharmacology effect or expression of new drug efficacy-pharmacology effect of CoQ_{10}.

CoQ_{10} has been validated to be associated with oxidative phosphorylation in cellular mitochondria, activating cell respiration and accompanying it elevation of ATP production while playing an important role in activating various body tissues.

Said CoQ_{10} is currently being utilized as a drug for oral administration, and when absorbed in animal bodies including that of human, it can activate the heart muscle so that it has been practically applied as an improving agent for the circulatory system aiming at treating angina pectoris. However, the current situation is that it is utilized virtually only for diseases with relatively early and light symptoms with respect to "light and medium degrees of edema, pulmonary congestion, pulmonary swelling and angina pectoris during congestive heart failure while conducting basic treatment" in clinical application. This is because CoQ_{10} is insoluble in water and its absorption from the digestive tract is extremely difficult such that a clinically effective blood concentration could not be reached. In fact, when CoQ_{10} was orally administered at a dose of 300 mg to 3 healthy males, the blood concentration 2 h after administration reached a maximum value of mere 3.4 μg/ml [Oyo Yakuri (Applied Pharmacology), 6(4), 695-706 (1982)]. As such, despite the excessively large dose compared with the actual clinical dose (10 mg dose, 30 mg daily), the maximum blood concentration is very low and the time to reach the maximum blood concentration is delayed, making it very inappropriate for treating an emergency disease such as congestive heart failure. Also, when CoQ_{10} is utilized as a liquid preparation, CoQ_{10} usually is dissolved in certain kinds of surfactants, but surfactants damage biological membranes so that side effects are expressed, making it inappropriate also.

The present invention solves the aforementioned problems of conventional CoQ_{10} by dissolving water-insoluble CoQ_{10} via inclusion in DM-β-CyD. When CoQ_{10} is administered in the form of an injection preparation or an oral preparation by dissolution via inclusion in DM-β-CyD, maximum blood concentration undoubtedly can be expected to be reached rapidly and the maximum blood concentration undoubtedly can be increased compared with that of the conventional CoQ_{10} preparation. Furthermore, the side effects experienced with adding surfactants can be avoided.

The CoQ_{10} utilized as medication is extremely expensive from the viewpoint of its production method, so that minimizing its dose via improving the absorption rate or choosing an administration route such as an injection preparation is an important task for economic reasons.

Based on such viewpoints, CoQ_{10} preparations intended for improving the absorption rate were disclosed (Japanese Patent Nos. Sho 54[1979]-92616, Sho 57[1982]-75916). However, these techniques either had limited applicability or had only marginal improvement in the absorption rate such that none is sufficiently satisfactory.

On the other hand, inclusion with cyclodextrin was proposed as a dissolving method for insoluble compounds, and inclusion with quinone compounds is also a known technique [Chem. Ber., 92, 378 (1957)], but the dissolution effect has not yet reached the level of clinically effective concentration.

The present inventors focused on the aforementioned situation and discovered that, when inclusion and dissolution of CoQ_{10} in DM-β-CyD were conducted, the solubility was increased more than tenfold compared with that in β-cyclodextrin, and that better treatment effect could be expected from an injection preparation. Furthermore, it was found that when the inclusion product of CoQ_{10} was administered as a liquid preparation or solid preparation by oral administration, the absorption rate was significantly increased, leading to improved bioavailability of CoQ_{10} and better clinical application.

Also, the presence of CoQ_{10} by inclusion in DM-β-CyD is evident from measurement by differential scanning calorimetry (DSC).

In other words, the aqueous solution of the inclusion product of CoQ_{10} in DM-β-CyD produced in the manner as shown in Application Example 1 below was freeze-dried in the manner as shown in Application Example 2, and the obtained powder (containing 1.96% CoQ_{10}) showed an exothermic peak (irreversible) at 151°C as shown in Figure 1, whereas in the case of CoQ_{10} alone and in the case of adding CoQ_{10} to freeze-dried product of DM-β-CyD and mixing gently in a mortar, an endothermic peak was observed at 48°C, respectively, as shown in Figure 2 and Figure 3, while no exothermic nor endothermic peak was observed for DM-β-CyD itself as shown in Figure 4 for reference. The following Table 1 summarizes these results.

Table 1

① 試　料	② 吸熱点	③ 発熱点
④ 本発明のC_{60}のDM-β-CyD包接体	—	151 °C
C_{60}	48°C	—
⑤ C_{60}とDM-β-CyDの混合物	48°C	—
DM-β-CyD	—	—

Key　1　Sample

2 Endothermic peak
3 Exothermic peak
4 Inclusion product of CoQ_{10} in DM-β-CyD of the present invention
5 Mixture of CoQ_{10} and DM-β-CyD

The above results revealed that CoQ_{10} exhibited an endothermic peak at 48°C corresponding to melting and an endothermic peak at 48°C was also observed for CoQ_{10} in the mixture of CoQ_{10} and DM-β-CyD while the endothermic peak disappeared from the inclusion product of CoQ_{10} in DM-β-CyD of the present invention, validating an interaction between CoQ_{10} and DM-β-CyD, i.e., the formation of an inclusion product.

In recent years, much attention has been focused on the function of mitochondria in experiments at cellular level and, in particular, the inhibition of endotoxin that affects energy production; whether it is a direct effect of endotoxin or it is caused by anoxia accompanying shock is not yet elucidated.

Establishing an experimental system for assessing the preventative effect or activating effect of CoQ_{10} on said inhibitory effect is difficult due to the water-insoluble nature of CoQ_{10}, so that validation is impossible. Because inclusion and dissolution of CoQ_{10} in DM-β-CyD succeeded in the present invention, an experimental system could be established and the cellular activating effect of aqueous solution of inclusion product of CoQ_{10} in DM-β-CyD was validated. From the result of this basic experiment, expressions of new treatment effects that were not possible, for example, activating effect on cerebral metabolism, improving effect on ocular fatigue and improving effect on liver function can be expected via administration of injection preparation, eye drop preparation and oral preparation of the present invention. Accordingly, the purpose of the present invention lies in providing therapeutically effective injection preparations, liquid oral preparations and solid preparations of CoQ_{10}.

According to the present invention, inclusion and dissolution of CoQ_{10} can be achieved as in the following.

In other words, DM-β-CyD is dissolved in water and while shielded from light, CoQ_{10} is added slowly and dispersed in said solution using ultrasound, followed by dissolution through agitation. This dissolving operation may be conducted at room temperature or by heating at, for example, 30-40°C. The time required for agitation is 2-24 h, preferably 4 h. After agitation the solution is filtered through a membrane filter (pore size 0.22 μm) to give an aqueous solution of inclusion product of CoQ_{10} in DM-β-CyD. The aqueous solution of inclusion product of CoQ_{10} in DM-β-CyD so produced is diluted to an effective concentration by adding water and formulated as drug preparations. Also, the aqueous solution of the inclusion product may be freeze-dried to prepare powder and stored. The powder is dissolved in water when used, which is

applicable as an injection preparation or as a liquid preparation for internal ingestion. The absorption rate of CoQ_{10} (blood concentration) of the freeze-dried powder by oral administration is significantly increased compared with that of CoQ_{10} alone.

Injection preparation, internal ingestion preparation, powder preparation, fine powder preparation and granule preparation may be cited as the examples of the drug preparations. When producing the drug preparations, conventional drug excipients, diluents, stabilizers, and desirable pH-adjusters and osmotic pressure regulators may be added.

Acute toxicity test of inclusion product of CoQ_{10} in DM-β-CyD

The acute toxicity was tested by orally administering powder of the inclusion product of CoQ_{10} in DM-β-CyD produced in Application Example 2 to ICR/JCL (Japan Clare) mice of about 20 g body weight, 6 per group, and observing the fatal cases after 7 days and determining the LD_{50} from the fatal cases.

The result revealed that the inclusion product of CoQ_{10} in DM-β-CyD of the present invention is very safe without causing fatality even at a large dose of 10 g/kg.

The aqueous solution of the inclusion product of CoQ_{10} in DM-β-CyD produced by the method of the present invention is stable against light and heat.

As shown above, inclusion and dissolution of water-insoluble CoQ_{10} in DM-β-CyD is conducted in the present invention and the production method is epochal, rendering it an excellent industrial production method. Furthermore, this inclusion product of CoQ_{10} in DM-β-CyD is a water-soluble powder so that its administering method is versatile and the application field is broadened.

Next, the present invention is described using the following experimental example and application examples.

Experimental Example 1

Table 2 shows the solubility of CoQ_{10} in aqueous solution of DM-β-CyD. CoQ_{10} 30 mg were slowly added to 5%, 10% and 15% aqueous solutions of DM-β-CyD, respectively, and dispersed via ultrasound, followed by agitating for 4 h in a water bath at 22°C while shielded from light, which was then filtered through a membrane filter (pore 0.22 μm), and the filtrate was quantified by determining the absorbance of maximum absorption near 275 nm wavelength.

Table 2. Solubility of CoQ_{10} in DM-β-CyD

① 溶解液	$Co Q_{10}$ の溶解度 ②
水 (対照) ③	0
④ 5%DM−β−Cy D水溶液	0.072
10% 〃	0.145
15% 〃	0.209

Key 1 Dissolved solution
2 Solubility of CoQ_{10}
3 Water (control)
4 5% aqueous solution of DM-β-CyD

Application Example 1

DM-β-CyD 100 g was dissolved in 1 L water, to which CoQ_{10} 1 g was added slowly and dispersed via ultrasound, followed by dissolution through agitation for 4 h in a water bath at 22°C while shielded from light, which was then filtered through a membrane filter (pore 0.22 μm), to give an aqueous solution of inclusion product of 0.1% CoQ_{10} in DM-β-CyD.

Application Example 2

The aqueous solution (about 1 L) of the inclusion product obtained in Application Example 1 was freeze-dried to give about 100 g powder of inclusion product of CoQ_{10} in DM-β-CyD.

Application Example 3

The aqueous solution of the inclusion product obtained in Application Example 1 was sterilized, followed by filling vials at 10 mL each vial under sterilized conditions and freeze-drying (10 mg CoQ_{10}/vial). The vial was then filled with nitrogen gas and stored after sealing.

Application Example 4

Sodium chloride 0.66 g was added to 100 mg aqueous solution of the inclusion product obtained in Application Example 1, followed by dissolution through agitation. The solution was then filtered and sterilized to prepare an injection preparation of 0.1% inclusion product.

Application Example 5

Saline solution for injection 10 mL was added to the vial sealed with the freeze-dried powder of the inclusion product obtained in Application Example 3, which was applied as injection preparation after dissolution.

Application Example 6

Powder (50 parts) of the inclusion product obtained in Application Example 2, 48 parts lactose and 2 parts magnesium stearate were mixed to homogenize to prepare a powder preparation. Alternatively, they were granulated and sieved to prepare granule preparation by conventional dry granulation method.

Application Example 7

Powder (50 parts) of the inclusion product obtained in Application Example 2, 15 parts starch, 12 parts lactose, 20 parts crystalline cellulose and 3 parts methyl cellulose were mixed and kneaded to homogenize, followed by crushing-granulation, drying and sieving to prepare a granule preparation.

Brief description of the figures

Figure 1 is a diagram showing the test result of differential scanning calorimetry of the inclusion product of CoQ_{10} in DM-β-CyD of the present invention; Figure 2 shows the test result of differential scanning calorimetry of CoQ_{10} alone, and Figure 3 shows that of a mixture of CoQ_{10} and DM-β-CyD while Figure 4 shows that of DM-β-CyD, respectively.

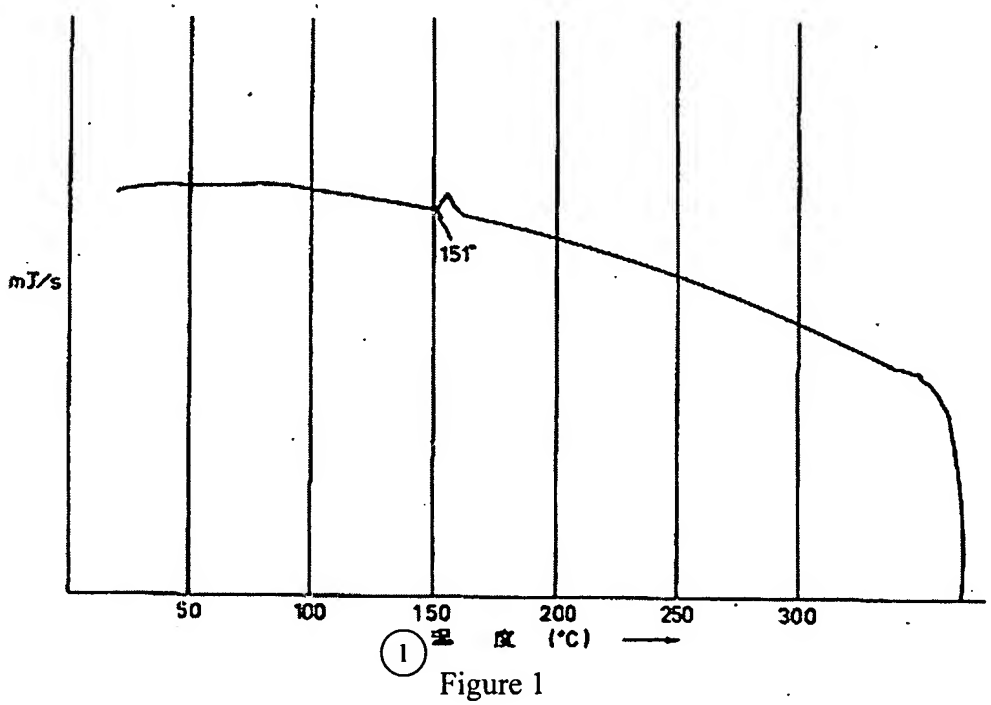

Figure 1

Key 1 Temperature (°C)

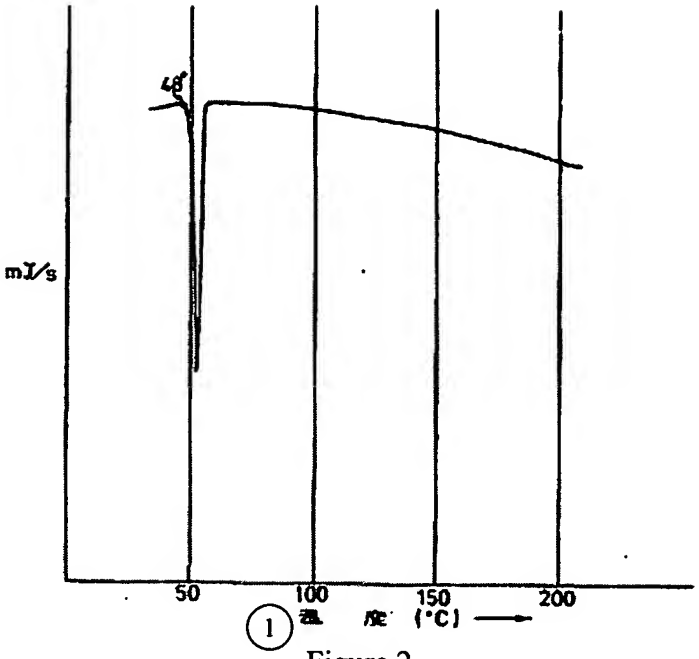

Figure 2

Key 1 Temperature (°C)

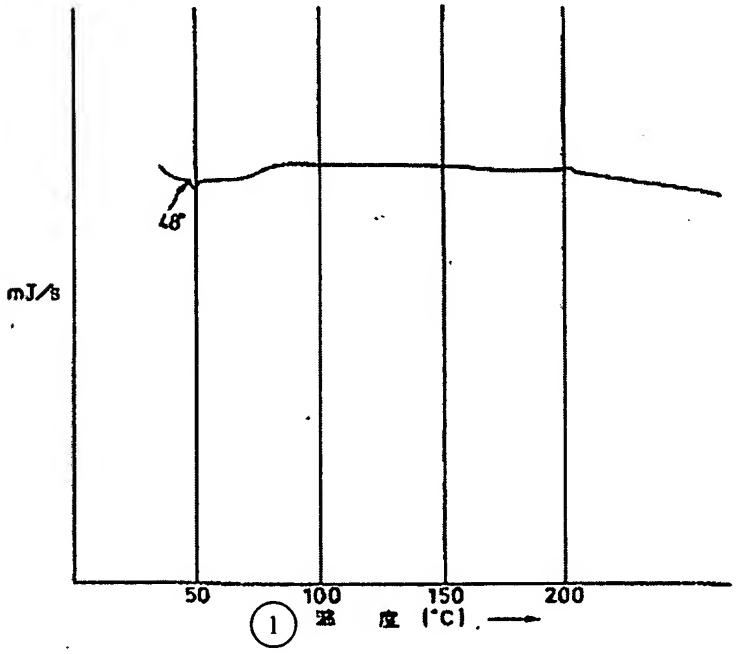

Figure 3

Key 1 Temperature (°C)

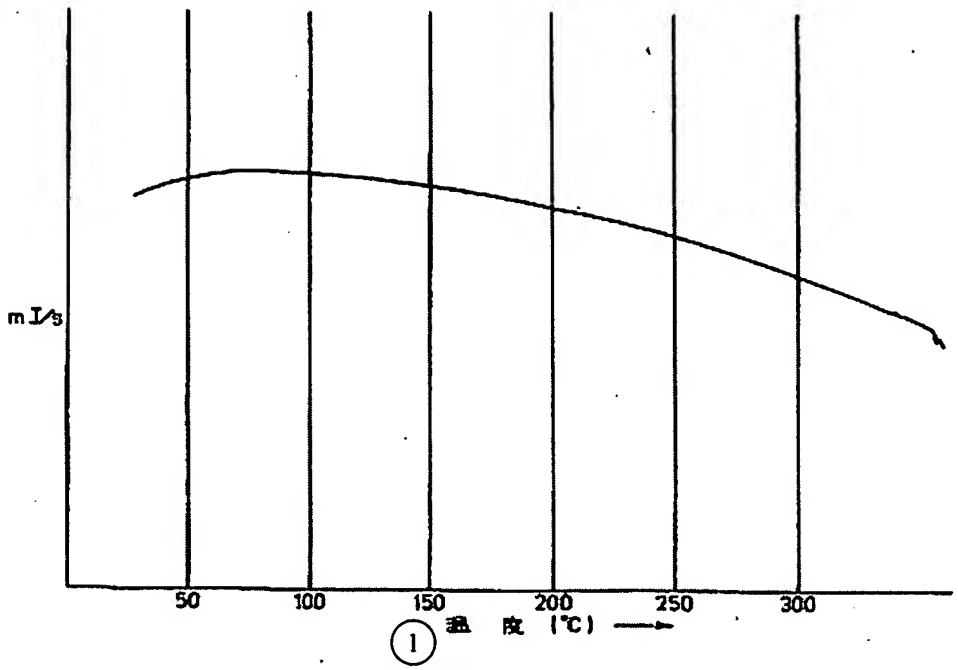

Figure 4

Key 1 Temperature (°C)